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## Short communication

## DNA adsorption measured with ultra-thin film organic field effect transistors

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## ABSTRACT

Organic ultra-thin film field effect transistors (FET) are operated as label-free sensors of deoxyribonucleic acid (DNA) adsorption. Linearized plasmid DNA molecules (4361 base pairs) are deposited from a solution on two monolayers thick pentacene FET. The amount of adsorbed DNA is measured by AFM and correlated to the concentration of the solution. Electrical characteristics on the dried DNA/pentacene FETs were studied as a function of DNA concentration in the solution. Shift of the pinch-off voltage across a wide range of DNA concentration, from very diluted to highly concentrated, is observed. It can be ascribed to additional positive charges in the semiconductor induced by DNA at a rate of one charge for every 200 base pairs. The sensitivity 74 ng/cm<sup>2</sup>, corresponding to 650 ng/ml, is limited by the distribution of FET parameters upon repeated cycles, and is subjected to substantial improvement upon standardization. Our work demonstrates the possibility to develop label-free transducers suitable to operate in regimes of high molecular entanglement.

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## 1. Introduction

A variety of biosensors have been developed for genomics and proteomics to monitor specific binding of biomolecules on solid-state substrates (Lyubchenko, 2004). Deoxyribonucleic acid (DNA) chips and DNA microarrays are used in molecular biology, pharmaceutical industry and clinical research to identify presence of specific biological targets. Fluorescence detection of labeled probes or targets is a widely used scheme for detecting specific recognition events (Hoheisel, 2006). Other methods include chemi-luminescence, surface plasmon resonance, quartz crystal microbalance and electrical conductivity (Drummond et al., 2003). Most of these methods lack a reliable quantitative response, which undermines their impact in early diagnosis or assessment of prognosis. Electronic transduction of the diagnostic event by low cost devices is desirable for in-field massive screening, without the need of sophisticated readout equipment or specialized personnel. For these applications, field effect transistors offer the advantage of a well-established scalable technology and ease of integration (Souteyrand et al., 1997).

Organic field effect transistors (OFET) are multi-parameter devices where charge carriers are generated and transported within the first few monolayers (ML) in contact with the gate dielectric

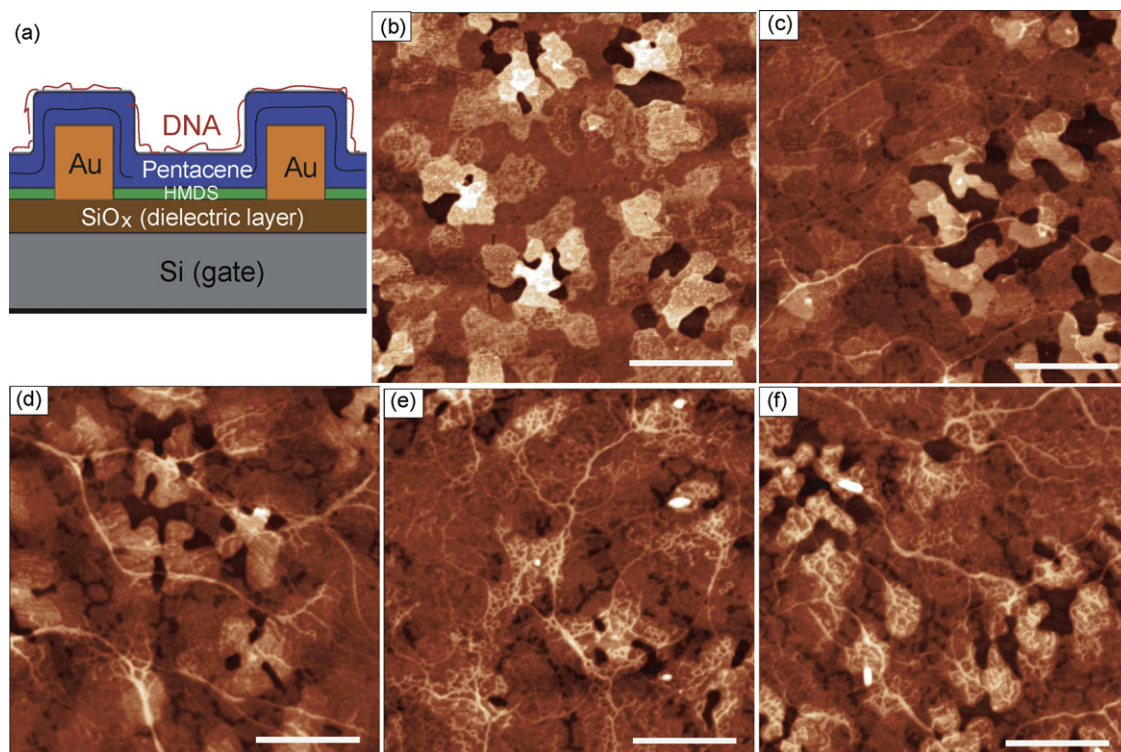
(Dinelli et al., 2004; Gomes et al., 2004). A schematic view of the device configuration is shown in Fig. 1a. In the case of ultra-thin film OFETs (1–5 ML), the exposure to the environment will affect the OFET response as molecules either adsorb on or approach the charge transport layer. Although this not desirable for the performance and stability of the device, it can become attractive for different applications. An example is the polyelectrolyte gate OFET (Said et al., 2006; Bernards et al., 2006), where the charge separation within a polyelectrolyte layer in contact with the organic semiconductor gates the charge carriers in the devices. As long as the faradic current within the polyelectrolyte is negligible, the response of the device reflects the change in the density of charge carriers induced by the double-layer. It can be envisioned that the capacitive coupling between large polyelectrolyte biomolecules adsorbed on the outer interface of an organic semiconductor will similarly modify the distribution of charge carriers. The electrostatic interaction of charge carriers and charged or polar species in the environment will be enhanced as their distance is small. Recently, OFET sensing DNA thick layers or highly concentrated solutions has been reported (Zhang and Subramanian, 2007; Stadler et al., 2007), although limited to the monitoring of the maximum on-current in the transfer characteristics.

Aim of the present work is to prove this concept using ultra-thin film transistors where the active layer consists of two pentacene monolayers only and the response is gated by a molecular polyelectrolyte, viz. DNA. We investigate the response of pentacene FETs upon the deposition of pBR322 DNA molecules from solutions of different concentration. We demonstrate the electrical

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**Fig. 1.** (a) Schematics of the OFET used in this work; (b) AFM morphology of pentacene film in the FET channel; each terrace is 1.5 nm high; (c–f) AFM images of DNA deposited on pentacene at different concentration: (c) 1 µg/ml, (d) 5 µg/ml, (e) 10 µg/ml and (f) 20 µg/ml. Scale bar for all AFM images is 500 nm.

transduction of DNA adsorption from low-coverage to highly entangled regime. DNA molecules induce an additional population of positive charges (holes) in the accumulation layer, contributing to the electrostatic field at the interface. The estimated sensitivity of the present devices is  $2.6 \times 10^{-14}$  mol/cm<sup>2</sup>, corresponding to about 160 pBR322 molecule/µm<sup>2</sup>. Our result opens interesting perspectives for a new class of label-free transducers of DNA adsorption without requiring binding agents or immobilization.

## 2. Experimental part

Our device is a pentacene field effect transistor whose active layer is made of two stacked molecularly ordered monolayers (Dinelli et al., 2004, see Appendix A). Each monolayer is 1.5 nm high. DNA is a suitable prototype biomolecule for this study, since it is a polyanion with homogeneous charge density along the chain ( $2e^-$ /bp), easy to visualize by scanning probe microscopy. As prototype, we use pBR322 plasmid (circular) DNA cleaved by a standard procedure at a precise position to yield DNA molecules of equal length (4361 bp) and sequence (see Appendix A). A drop of water solution (5 µl volume) whose DNA concentration (DNA) varied in the range between 1 and 20 µg/ml, and with constant buffer concentration of 10 mM tris-hydroxy-methyl-amino-methane (TRIS), is deposited on the transistors for 10 min. Then the drop is washed with UHQ water and dried with N<sub>2</sub> prior to perform electrical transfer characteristic measurements.

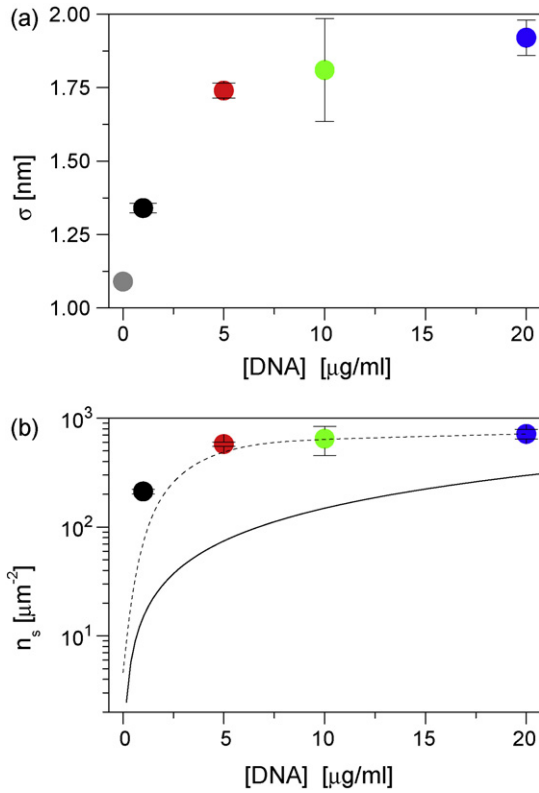
Pentacene bottom contact field effect transistors were made by high vacuum sublimation. The transistor layout, the fabrication process and the electrical characterization were described earlier (Stoliar et al., 2007). Channel length  $L$  and width  $W$  range from 10 to 40 µm and 1 to 20 mm, respectively. Pentacene coverage of 2.1 ML was chosen for all devices, so that the active channel is exposed to the environment (Gomes et al., 2004). Field effect response of these pristine transistors in air was measured as benchmark. For the devices with  $L = 30$  and 40 µm the charge mobility in ambient

extracted from the transfer characteristics in the saturation regime is  $0.014 \pm 0.003$  cm<sup>2</sup>/V s.

A differential measurement with respect to the bare pentacene and after contact with the buffer solution is carried out for every device. Each experiment involves three steps: (i) the OFET is fabricated and transfer characteristics acquired; (ii) a blank device is made by drop casting of the buffer solution (without DNA) on the device for 10 min, dried, and then characterised; (iii) DNA in the buffer solution is deposited and the device is measured again after drying it. The time lag between the drop deposition and the electrical measurement must be kept constant to 10 min because there is a small drift in time of the transistor response (i.e. the threshold voltage change with a rate of about 10 V/h).

## 3. Results and discussion

The morphology of DNA on pentacene is shown in Fig. 1. Pentacene stacked monolayers are clearly visible. Islands of the third and fourth monolayer are beginning to form above two almost complete monolayers, as commonly observed in several conjugated oligomer ultra-thin films. The effective coverage equals 2.1 ML, with an rms surface roughness of 1.1 nm. By comparison, surface roughness of the FET channel before sublimation of pentacene is 0.5 nm. Adsorption of linear DNA at low concentration (Fig. 1c) yields formation of bundles consisting of a few molecules. At higher concentration (Fig. 1d–f) a hierarchical DNA network is formed, where larger bundles are entangled with small DNA chains. The morphology does not seem to change substantially as strong entanglement is achieved at higher concentration (Fig. 1e and f). We monitor the morphological changes vs. [DNA] by measuring the length-scale saturated rms roughness  $\sigma$  (Palasantzas and Krim, 1995) from the AFM images acquired in the FET channels. The plot in Fig. 2a shows that  $\sigma$  first increases, as a result of increasing coverage and DNA bundling, then saturates at concentrations higher than 10 µg/µl. Saturation may be ascribed to the adsorbed DNA chains repelling



**Fig. 2.** Concentration dependence of (a) rms roughness  $\sigma$ ; (b) number density  $n_s$  of DNA molecules extracted from Eq. (3) (dash line is to guide eyes) with the predicted trend from the diffusion-controlled deposition Eq. (4) drawn as black line.

DNA molecules from the solution, as a consequence of depletion of electrostatic screening by the lack of buffer, or by the saturation of accessible adsorption “sites” on the pentacene surface. DNA in excess beyond a critical concentration does not bind to the surface and is easily washed away.

The number density  $n_s$  of DNA molecules adsorbed on the pentacene surface is related to the effective coverage:

$$\Theta_{\text{DNA}} \approx n_s \cdot \frac{\pi d L}{2}, \quad (1)$$

where DNA is approximated as a cylinder of molecular diameter  $d \sim 2$  nm and length  $L \sim 1.5$   $\mu\text{m}$ . The factor  $\pi/2$  accounts for half of the cylinder surface being closer to the pentacene surface. For each [DNA],  $\Theta_{\text{DNA}}$  is estimated from AFM images as

$$\Theta_{\text{DNA}} = \frac{\bar{h}_{\text{DNA}}}{d} = \frac{\bar{h}_{\text{Pen+DNA}} - \bar{h}_{\text{Pen}}}{d}, \quad (2)$$

$$n_s = \frac{2\bar{h}_{\text{DNA}}}{\pi d^2 L}, \quad (3)$$

where  $\bar{h}_{\text{DNA}}$  is the mean height of the deposit,  $\bar{h}_{\text{Pen+DNA}}$  ([DNA]) and  $\bar{h}_{\text{Pen}}$  are the mean heights measured on pentacene layers with and without DNA, respectively. The analysis is carried out by alignment of the respective topography histograms at the lowest peak representing the background (lowest terrace of the bare pentacene substrate). The plot of  $n_s$  vs. [DNA] is shown in Fig. 2b. The  $n_s$  increases linearly at low [DNA] then slows down at high [DNA]. This is consistent with the roughness trend shown in Fig. 2a. We compare the experimental points with the expectation of diffusion-controlled deposition (Lang and Coates, 1968):

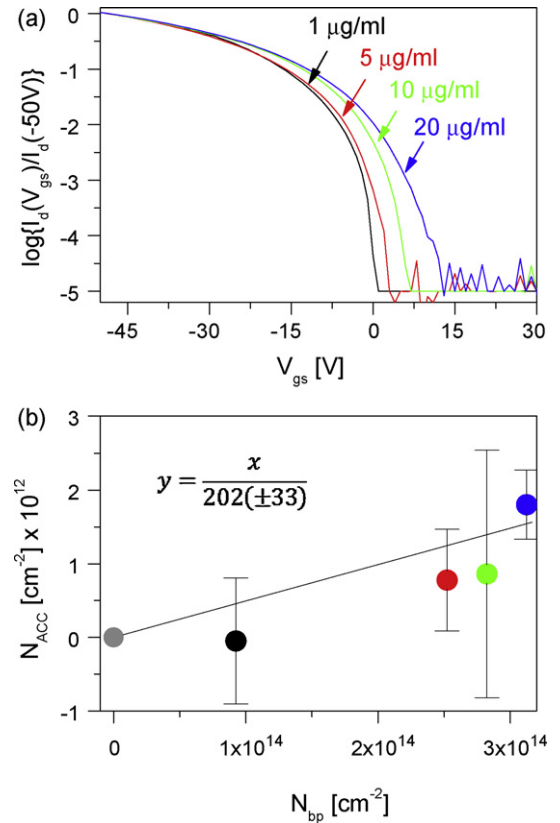
$$n_s = \frac{N_A \cdot [\text{DNA}]}{MW} \cdot \sqrt{\frac{4 \cdot D \cdot t}{\pi}}, \quad (4)$$

which is plot as a continuous line in Fig. 2b. Here  $D$  is DNA diffusion coefficient,  $N_A$  Avogadro's number, molecular weight  $MW = 2.83 \times 10^6$  Da, and the time  $t$  is constant in our experiment (10 min). The diffusion coefficient of DNA in water is taken as  $D = 5.4 \mu\text{m}^2/\text{s}$  (Shen et al., 2006). The marked deviation suggests that the mechanism is not diffusion-controlled, as possibly due to DNA bundling in solution or influence of capillary flow.

We now relate the variation of FET parameters to  $n_s$ . The transfer characteristics of pentacene/DNA in buffer in Fig. 3a exhibit a shift into depletion. The pinch-off voltage  $V_p$ , separating the depleted region at high positive voltages, and the sub-threshold region where the transfer current sets-in, shifts towards more positive voltages.  $V_p$  represents the value of the gate voltage at which all the positive charge carriers (including the parasitic ones due to doping states near the gate dielectric (Brown et al., 1999)) are expelled from the channel, thus annihilating the transfer current. A shift towards more positive values caused by DNA adsorption implies that the negative charge of the adsorbed DNA molecules induces an additional density of positive charges  $N_{\text{acc}}$  in the transport layer according to:

$$N_{\text{acc}}^{\text{DNA}} = \frac{V_p^{\text{DNA+buffer}} - V_p^{\text{buffer}}}{q} \cdot C_i \quad (5)$$

where  $V_p^{\text{DNA+buffer}}$  is the pinch-off voltage extracted from the measurements with DNA and buffer,  $V_p^{\text{buffer}}$  the pinch-off voltage extracted from the measurements with buffer, and  $q$  is the electron charge. Eq. (5) holds if  $d_s \cdot C_i \ll 2 \cdot \varepsilon_s \cdot \varepsilon_0$ , where  $d_s$  and  $\varepsilon_s$  are the thickness and dielectric constant of the pentacene layer,  $C_i = 19 \text{ nF cm}^{-2}$  the capacitance per unit area of the gate dielectric and  $\varepsilon_0$  the vacuum permittivity (Meijer et al., 2003). The extra holes act as electron acceptors as they need to be balanced by negative



**Fig. 3.** (a) Transfer characteristics for the transistors at different DNA concentrations (increasing to the right); (b) density of acceptor states introduced by the DNA (extracted from the shift in pinch-off voltage according to Eq. (4)) in relation with the number of DNA base pairs per unit area (estimated from Eq. (3)).



charges generated by the additional gate voltage in order to deplete the FET channel.

Fig. 3b shows the surface density of acceptor states induced by the DNA (from Eq. (5)) as a function of number density of base pairs  $N_{bp} = 4361n_s$  adsorbed on the FET channel. These data represent a statistical set of accumulated measurement averaged over 50 devices. The linear regression yields a slope of one acceptor state over  $202 \pm 33$  bp. This means that a single DNA molecule used in our study (1482.74 nm long) induces about 22 acceptor states. The minimum shift  $\Delta V_p$  detected above the fluctuations of repeated transfer curves is about 3 V, which corresponds to about  $7 \times 10^5$  bp/ $\mu\text{m}^2$ , or  $n_s \sim 160 \mu\text{m}^{-2}$ . From Fig. 2b this translates into concentration sensitivity of our pentacene FET devices in the order of 650 ng/ml. State-of-the-art of electrochemical DNA sensors exhibits a sensitivity of 8 ng/ml (Liu and Anzai, 2004). The 100 times difference is largely due to the dispersion of the values of the pinch-off voltage. Standardization and optimization of the organic FET response (e.g. reducing the dispersion of pinch-off voltage from 3 V down to 30 mV) would enhance substantially the sensitivity value aligning it with that of state-of-the-art inorganic semiconductor devices.

Other FET parameters are affected by the adsorption of DNA molecules. We observe a shift in the charge mobility vs. [DNA] from 50% to 20% of the value measured on the corresponding buffer samples. However, this trend is not as clearly defined as the trend of the pinch-off voltage. The pinch-off voltage appears in this case a more robust parameter for correlating DNA concentration in solution (or the number density of adsorbed DNA molecules) to the response of the device.

As DNA is a hygroscopic molecule, and a water solvation shell with positive counter ions will form on the surface, the trends observed indicate that the capacitive coupling dominating the effect on the device parameters comes from the negative charge in the DNA, and thus from the proximity contacts of the DNA molecules and pentacene interface. We do not observe drift diffusion of the DNA molecules across the fluid layer at the pentacene/air/DNA interface, which may either cover or hamper the possibility to measure quantitatively the amount of adsorbed DNA. This hints to the fact that DNA molecules are steadily binding pentacene, possibly via electrostatic interactions. All these FET data, although limited in number, are robust due to the large statistics, and show the possibility to directly correlate the FET parameters to the number density of adsorbed DNA molecules.

#### 4. Conclusions

Our work shows a first evidence of an organic field effect device detecting the presence of DNA molecules deposited from a solution onto the organic semiconductor ultra-thin film. The electrical response is correlated to the DNA concentration and specifically to the number of adsorbed DNA molecules. Currently our devices exhibit a sensitivity of about  $7 \times 10^5$  bp/ $\mu\text{m}^2$ , which is however subject to large improvements as device response is standardized. This is important in view of quantitative sensing schemes, based also on specific recognition mechanisms. Future sensing layouts based on organic FETs will require the development of a hybrid bio-organic technology where the solution containing biomolecules is integrated in the FET by means of a microfluidics device.

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#### Appendix A

##### A.1. Materials and methods: DNA

Single length DNA has been prepared by endonuclease restriction enzymatic reaction on plasmid. 30  $\mu\text{l}$  solution of circular DNA pBR322 (FERMENTAS, SD0041) at 0.5  $\mu\text{g}/\mu\text{l}$  has been digested with BSP68I restriction enzyme 30  $\mu\text{l}$  at 10 u/ $\mu\text{l}$  in a buffered solution of  $10\times$  Buffer O diluted 1:8. The solution has been incubated  $37^\circ\text{C}$  in thermostatic bath for 10 h. The restricted DNA has been recovered by Nucleo Bond Kit (Macherey Nagel).

Droplet of 5  $\mu\text{l}$  solution of linearized DNA plasmid (pBR322, 4361 bp, FERMENTAS #SD0041, M-MEDICAL SRL, Milano, Italy) in 10 mM tris(hydroxymethyl)aminomethane buffer (TRIS #93377, FLUKA, SIGMA ALDRICH SRL, Milano, Italy) was deposited for 10 min on freshly evaporated pentacene (#45797, FLUKA, SIGMA ALDRICH SRL, Milano, Italy) on FETs. Then the drop is washed with UHQ water and dried with  $\text{N}_2$  prior to perform electrical transfer characteristic measurements. Concentration of DNA varied from 1, 5, 10 to 20  $\mu\text{g}/\text{ml}$ .

##### A.2. Morphological analysis

AFM images were performed by stand-alone SMENA NT-MDT microscope in semi-contact mode in air using Super sharp diamond-like carbon (DLC) tips (NSG01.DLC, NT-MDT, PRA.MA. Sondalo, Italy) with typical resonant frequency 150 kHz and force constant 5.5 N/m.

The Roughness Analysis mode of Image analyses software 2.1.2 (NT-MDT, PRA.MA. Sondalo, Italy) calculates the basic statistical parameters for the source object (2D function) and forms the function values distribution density histogram. Root mean square roughness rms is in accordance with ISO4287/1.

##### A.3. Details of electrical measurements

Electrical characteristics were recorded with Keithley 6430 and 487 electrometer and source-measure unit, with automated acquisition of the transfer characteristics (gate sweep). Our transistors were operated in saturation using a drain-source voltage  $V_{ds} = -21$  V.

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